“Comparative study of two Moringa species using Omics approaches”

BY

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Synopsis

Plants have been used as dietary supplements and as part of traditional treatment regimens for a variety of diseases for hundreds of years. Moringaceae is a plant family with 13 species, two of which are native to India, *Moringa oleifera* Lam and *Moringa concanensis* Nimmo (Olson 2002). *M. oleifera* has received more research than the other species in this family, and it is now cultivated all over the world. The enormous health and nutrition benefits of this plant, as well as its ability to thrive in drought conditions, make it a valuable crop. *M. concanensis*, a closely related species found in India, mainly in the Konkan region, has long been used as a medicinal plant (Padayachee and Baijnath 2012). Specific plant parts used in traditional medicine can be evidenced scientifically using transcriptome data and chemical identification based on mass spectrometry (Upadhyay et al. 2015; Pasha et al. 2020; Naika et al. 2022). Several groups have reported the *M. oleifera* genome and transcriptome, as well as the presence of biologically active molecules in various plant parts (Vergara-Jimenez, Almatrafi, and Fernandez 2017; Tian et al. 2015; Shyamli et al. 2021). *M. concanensis*, on the other hand, has received little attention in terms of sequencing and chemical identification. As a plant native to India, research on *M. concanensis* will aid in its preservation and will reveal the medicinal and nutritional benefits of various parts of the plant. It is essential to step up research on these less-known species so that they receive the attention they deserve and contribute to their preservation and recognition as a natural resource.

The aim of this thesis was to present the first report of *M. concanensis* transcriptome and a comparison with *M. oleifera* transcriptome. This was accomplished through the use of NGS techniques and computational methods, supported by metabolite analysis and *in vitro*

assays. In a recent study, transcriptome profiling was used to investigate the candidate genes involved in the biosynthesis of selected secondary metabolites, vitamins, and ion transporters from *M. oleifera* (Pasha et al. 2020). The current *M. concanensis* study serves as a transcriptome and metabolome repository, revealing similarities and differences to *M. oleifera*. The thesis narrowed its focus to diabetes-related questions by using the data generated from both plants. Diabetes is a chronic metabolic disorder that poses a serious risk to human health and has garnered global attention (Gheith et al. 2016). Unlike *M. concanensis*, the antidiabetic properties of *M. oleifera* have been studied extensively (Mbikay 2012). Recently, a study on mice demonstrated the antidiabetic properties of *M. concanensis* (Balakrishnan, Krishnasamy, and Choi 2018). Several small molecules have been studied for their anti-diabetic properties, and many of them have proven to be effective and are currently in use. In comparison to currently available molecules, natural products may prove to be a more effective and benign for diabetes treatment (Vasudevan and Garber 2005). Three major compounds important for these plant antidiabetic properties were chosen based on previous reports. Transcriptome data from *M. concanensis* and *M. oleifera* tissues were used to investigate the expression of their biosynthesis enzymes. Both species were then compared using metabolite profiling and *in vitro* experiments to look into their potential anti-diabetic activity. In addition, the genes responsible for drought tolerance in the *M. oleifera* plant were investigated. The *M. oleifera* genome and drought-induced transcriptome data from a recent study (Shyamli et al. 2021) aided in the investigation of the promoter region of differentially expressed genes under drought stress.

The thesis is divided into six chapters. **Chapter 1** of this thesis gives a general overview of *Moringa* species and their therapeutic benefits. The chapter also discusses the computational methods, applications, databases, web servers, and experiments used in this thesis. The thesis objectives and outline have been summarised at the end. **Chapter 2** discusses the sequencing of the *M. concanensis* transcriptome and its relationship to the *M. oleifera* transcriptome. RNA sequencing, transcriptome assembly, downstream analysis, and estimating the abundance of each transcript are all covered in this chapter. The gene families of both species were compared to those of closely related species and species-specific gene families were analysed. **Chapter 3** explains metabolite analysis and the expression of enzymes involved in the biosynthesis of three potential anti-diabetic compounds found in various tissues of *M. oleifera* and *M. concanensis*. The estimated expression data from the *M. concanensis* transcriptome were validated further using RT-qPCR. The compounds were further quantified in crude leaf extract of both species. **Chapter 4** of this thesis investigates the inhibitory activity of crude leaf extract

from both species on the digestive enzymes α-amylase, α-glucosidase, and Dipeptidyl peptidase-4 (DPP-4). These enzymes are important targets for regulating blood glucose level. Separately, the inhibitory activity of Benzylamine, a significant plant-specific substance thought to mediate the plant hypoglycaemic activity, has been investigated. In addition to various assay studies, the toxicity of this compound was investigated. Through *in vitro* experiments, this chapter sheds light on the antidiabetic activity of leaf tissue from both plant species. In addition to the health benefits of *M. oleifera*, as an important crop, it is critical to investigate the plant drought stress tolerance mechanism. **Chapter 5** of this thesis discusses the identification of drought-stress response genes from *M. oleifera* and the analysis of *cis*-elements in the promoter region. This chapter outlines the transcription factors and their binding sites in the promoter region that are important for the drought-stress tolerance of *Moringa* species. Finally, **Chapter 6** of this thesis provides a summary of overall work and also provides future directions to this thesis.

Collectively, the thesis discusses the transcriptome profiling of *Moringa* species, explores potential anti-diabetic compounds present in these species, determines inhibitory activity to study antidiabetic activity, and finally look into the genes involved in the response to drought stress. The transcriptomes of both species were used to estimate the expression of important enzymes in different tissues. The analysis revealed the importance of leaf tissue in antidiabetic activity. In experiments, the leaf tissue demonstrated significant inhibitory activity against important antidiabetic targets in the gastrointestinal tract. The metabolites profiling of leaf tissue and the assay studies on compounds like benzylamine led to the conclusion that the presence of such compounds in the tissue could be explained by its high activity. Furthermore, the genome and transcriptome enabled to investigate drought stress response genes and their promoter region in *M. oleifera* plant. Overall, this study not only provides transcriptome resources for *Moringa* species, but also sheds light on antidiabetic potential of both species. The knowledge gained from this investigation of the *M. concanensis* plant will be helpful for future research into a variety of diseases and also contribute to the conservation of plant.

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